The microbial partners of stingless bees and their hives

I saw in a documentary that beehives contain beneficial microbes, is that true?

Photo: D. Burge

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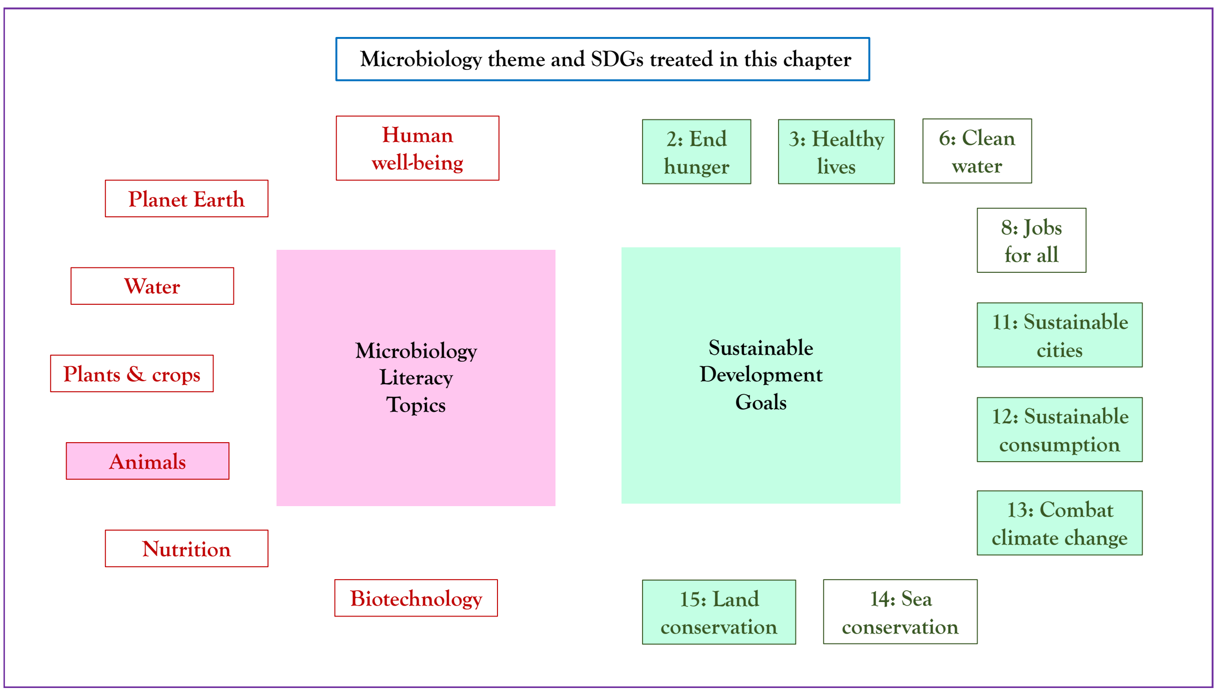
Storyline

Stingless bees, the lesser-known relatives of honeybees, get their name from their reduced sting. Due to their sweet honey with healing properties, humans have interacted with these bees since ancient times. There are approximately 500 described species, and the group is widespread in tropical regions of the world. Around 75% of this diversity is found in the neotropics, which consists of Central and South America, including the tropical southern part of Mexico and the Caribbean.

Stingless bees are eusocial insects characterized by a reproductive division of labor, overlap of generations, and cooperative nesting, and are considered important pollinators. The bees and the different components of their colonies are associated with microorganisms that influence their development and even the evolutionary success of the species. Unfortunately, as is the case for many neotropical species, bees are being threatened by climate change, habitat loss, habitat fragmentation and pollution by agrochemicals. Therefore, urgent action is needed not only to conserve them for the benefit of the planet but also to keep learning from their lifestyle and relationships with beneficial microbial partners.

The Microbiology and Societal Context



The microbiology: the hive of stingless bees; strategies to maintain a healthy hive; the stingless bee microbiome; diversity; functions of the microbiome in the stingless bee colony. *Sustainability issues:* threats to the bee microbiome and the potential use of beneficial microbes to combat them; honey: one of the stingless beehive components with beneficial properties**;** pollination; beehive model of communication.

The microbial partners of stingless bees and their hives: The Microbiology

1. ***The hive of stingless bees.*** Stingless bees form permanent colonies of varying sizes, ranging from hundreds to thousands of individuals. Normally, the colonies are formed by two female castes (groups of individuals specialised to perform certain tasks): the workers and the queens, and one male caste, the drones. These insects are highly social because they have a clear division of labor and distribution of reproductive duties. The workers are altruistic since they take care of the young without receiving any direct benefit. Within the hives, we can find the brood chambers, which are small chambers made of soft cerumen (wax and resin), each built to rear one individual bee. In short, before the queen lays an egg, several workers regurgitate brood food into the chamber and, after the egg is laid, the chamber is closed and remains sealed until the adult bee emerges. Around the brood chambers we can see the involucrum, a resistant material made of different proportions of wax and resin. To cover the walls of the nest and to create a well-protected space, the bees produce a stronger mix called batumen, which has more resin, mud, plant material, and in some species, even animal faeces. Around the involucrum are the storage pots, filled with honey or pollen, where nectar, sugary secretions produced by sap-sucking insects or fruit juice are converted into honey. The process of making nectar into honey requires water evaporation, fermentation by microbes, including yeast and bacteria, as well as the addition of enzymes (proteins that speed up the chemical reactions of cells) and other substances secreted by the worker bees' glands.

Photo: E. Herrera, D. Burge y M. Ramírez.

2. ***Strategies to maintain a healthy hive.*** How do these bees keep their hive healthy when so many are inside a humid and warm environment? For instance, workers leave the hive to find food, and could bring harmful microorganisms back with them. A component that plays a leading role in protecting the colony from disease is resin. The bees obtain this sticky substance from

plants and use it in nest construction, food storage and defence against predators and disease-causing microbes known as pathogens. Resins have antimicrobial properties that prevent food from going bad and pathogens from attacking it, which is very important in tropical environments (where microbes multiply more easily) and in social insect communities (diseases can spread rapidly within a dense population of genetically related individuals). Some studies also report that resin can help shape and support beneficial microbial communities that are essential to the colony's performance. Moreover, the use of resin affects not only the hive's microbial composition, but also the bees' intestinal microbial diversity and even their physical properties and learning abilities.

3. ***The stingless bee microbiome.*** Insects, like other organisms, are considered holobionts: individual metaorganisms consisting of the insect plus its microbes. Furthermore, the collection of genomes of the microorganisms associated with the stingless bee, known as the microbiome, helps the insect in many ways, such as in nutrition, digestion, toxin resistance, and defence. The benefits that insects obtain from of their microbiomes have contributed to their diversification and successful adaptation to different environments through time.

The Meliponini (stingless bee) intestinal microbiome, although diverse in its composition, generally includes bacteria, yeasts, fungi, and viruses. These microbial symbionts, found in the bees' guts, are intimately associated with the bees, and evolve with them. The microbiome's composition is not static: it changes depending on the life stage of the bee and its role within the colony, as well as the species or the community (due to diet, genetic information, and the environment). Scientific studies have shown that host sociality facilitates the development and maintenance of specialized microbiomes; some components of the microbiome could be passed horizontally from maternal nests to daughter nests when the worker bees transport building materials and stored food to a new nest.

Knowledge about the microbiome of stingless bees is still limited. A previous study of the gut microbial diversity of 25 species of corbiculates (honeybees, stingless bees, and bumblebees) shows that one member of the core microbiome (set of microbial taxa that are characteristic of a host or environment) is *Lactobacillus*, which has important functions such as protection against pathogens, food digestion, and pollen fermentation to make beebread.

4. ***Diversity and functions of the microbiome in the stingless bee colony.*** Inside the hive, the bees are not the only ones who have beneficial microorganisms: the different components of the colony also have this valuable help. Although there is some knowledge about the functions of certain microorganisms in some stingless bee species, existing research is very specific, which is why further research is needed to obtain a general perspective of microbial associations in all Meliponini.

Stingless bee colonies are generally colonized by three major groups of bacteria with different functions: *Bacillus*, which helps ferment honey and create bee bread, a mixture of nectar and pollen used by bees as food for their larvae; *Streptomyces*, which secretes antibiotics (substances that destroy or inhibit bacterial growth) into the brood chambers to protect the larvae from pathogens; and lactic acid bacteria which, just like in the bee's gut, seem to provide both services, protection against pathogens and pollen fermentation. These bacteria can enter the hive from different sources including pollen, the air, flowers, and the digestive tracts of the bees themselves.

These microorganisms can also be beneficial to humans; for example, many of these bacteria have been found to be potential biocontrol agents or biofertilizers, as well as probiotics, which are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host.

The most common genus of yeasts found in stingless bees' colonies is *Starmerella,* which, together with bacteria, contributes to the fermentation of nectar stored inside cerumen pots. Furthermore, a species of *Zygosaccharomyces* that grows inside the brood cells seems to help with larval survival and development to the adult stage by producing compounds that play an essential role in the regulation of cell functions.

The roles of filamentous fungi and viruses within stingless bee colonies have not been extensively studied yet. However, it is believed that fungi, apart from being potential opportunistic pathogens, could serve as a food resource for the bees (although the fungal spores are much less nutritious than pollen). Most of the viruses are believed to be harmful or indicators of unhealthy bees, but further studies are needed to determine their roles in nature and which ones can be found in healthy bees.

5. ***Threats to the bee microbiome and the potential use of beneficial microbes to combat the threats.*** The microbiome is crucial to bee health, but its composition can be altered by changes in diet or external stressors like agrochemicals or changes in regional temperature and precipitation. In the case of worker bees (which can be considered the backbone of the colony), antibiotics used to treat larvae in the hive as well as pesticides can perturb their microbiomes, which makes them more vulnerable to pathogens. For this reason, the use of beneficial microbes (such as the ones used in probiotics) to improve colony health in social bees is gaining increased attention. The problem is that most, if not all, current bee probiotics on the market consist mainly of non-bee bacteria (being more like human probiotics). It is important to note that studies have shown that the probiotics needed by bees have a greater impact on immunity or capacity to resist infection when they are composed of species from their guts. This is because these microorganisms can re-establish and persist in the bees and have effects similar to their own microbiomes, unlike commercial formulas which don't establish well. How to solve this problem? The most straightforward approach would be introducing commercial bee probiotics composed of bee´s microorganisms. Another strategy considered to be potentially beneficial is the use of engineered probiotics, genetically modified bacteria from bee´s gut that are enhanced to have specific functions, such as degrading harmful substances, helping the bee gain resistance to agrochemicals, or even directly improving immunity.

6. ***Honey: one of the stingless beehive components with beneficial properties.*** One of the most important components produced by stingless bees is honey. More than two thousand years ago, the ancient Mesoamerican Mayan culture started keeping stingless bees and used their honey in food, religious objects and medicine. Presently, a series of investigations performed with the honey of the Costa Rican species *Tetragonisca angustula* has allowed this highly regarded traditional medicine to become a novel candidate component of wound dressings.

Microorganisms infecting wounds can aggregate to form protective covers called biofilms to defy a host's immune system and to resist antibiotic treatment, including those used to prevent or inhibit the growth of microorganisms that cause skin infections. Biofilms thus impair the process of wound healing. However, *T. angustula* honey can destroy bacterial biofilms. The antibiofilm active ingredients are two proteins, named the *T. angustula* biofilm destruction factors (TABDFs).

Stingless bees can also be a novel source of antibiotics. Recent findings suggest that some of the microorganisms that live with these bees produce antimicrobials that could contribute to creating new medicines and new strategies for treating infections.

7. ***Pollination.*** In flowering plants, the transfer of pollen, which contains the male reproductive cells, from the male sex organ (anther) to the receptive portion of the female sex organ (stigma) often needs external agents like wind, water, or animals. Stingless bees collect pollen from anthers and transport it to the nest in a special pollen basket on the bees' hind legs. Pollen is one of the main nutrient sources for the colony and is placed in cells of the wax comb where the workers add secretions made up of beneficial microorganisms, enzymes and honey. The pollen is then fermented by bacteria (lactic acid bacteria and *Bacillus*) to produce bee bread, the main source of proteins, lipids, vitamins, and macro and microelements in their diet.

Although much of the pollen ends up in the nest, some of it may be transferred from one plant to another and result in pollination. This process benefits both plants and humans since bees pollinate a large proportion of our food crops, and about 50 species of bees are specifically bred for this purpose. They thus contribute to fruit and seed diversity, and maintain wild plant populations, thereby supporting biodiversity and the functioning of ecosystems. It has been found that wild bees are very important in crop production; without them, 5-8% of all crops could be lost, meaning humans would have to implement changes in their diet and would need to vastly expand the land dedicated to supply the rising demands in crop production.

The decline of honeybee populations has been widely reported. However, although documentation of biodiversity loss in wild bees in tropical regions has begun, the decrease of these species has remained mainly unnoticed, which is worrying since stingless bees are key members of tropical forests.

8. ***Stingless bees teach us how to communicate efficiently***. Bees have a great capacity for coordination: they use complex and interactive communication systems to organize their daily activities, such as working together to collect food, defend the colony and reproduce. For example, for recruitment during food collection, some species bump (jostle) each other to communicate the presence of resources, while other species emit thoracic pulses (buzzing sounds) which can report a food source and its distance. Moreover, in some species the foragers (which oversee the finding and gathering of food) deposit scent marks (pheromones) on the route from the food source to near the nest entrance to alert other foragers; these can be attractors or repellents depending on what the bees want to communicate. Additionally, researchers are investigating whether this communication is maintained or even improved by microorganisms (mainly from resins) on the bees' outer layer, known as the cuticle, which helps them recognize each other.

Although there can be hundreds to thousands of bees inside a colony, all of them can work together cooperatively and in harmony. How do they manage this without conflict? A colony is not a rigid unit where the queen rules and the workers obey. Eusocial bees have at least two levels of organisation, the individual and the colony level. The individuals (the workers) make decisions based on their experiences and the immediate information the environment provides them, which is enough for the hive to work as a unit.

We can thus compare the beehive to human societies; the hive can be seen as a society where each member has an essential role in the good performance of the community, achieving synchronization through communication. Therefore, for a society to be successful, each individual must take responsibility and maintain adequate communication with the other members.

Relevance for Sustainable Development Goals and Grand Challenges

Goal 2: End hunger, achieve food security and improved nutrition and promote sustainable agriculture: Bee products and their microorganisms have been used for human consumption since ancestral times and are still important nowadays. Although honey is the most widely known product, other components such as propolis and pollen are also edible and possess various properties beneficial to human health. Since stingless bees are less known, the introduction of their colony components into modern societies could help bring forth new markets and healthy products. Also, the management of Meliponini colonies could improve pollination and increase crop yields.

Goal 3. Ensure healthy lives and promote well-being for all ages: Consumption of bee products, including the microorganisms they contain, is known to be beneficial to humans. Therefore, bee products such as honey and propolis can be used as home remedies for minor ailments; they can also be developed as medicines, functional foods, and high-end cosmetics for tissue regeneration.

Goal 11: Healthy environments: Human activities produce pollutants, the amounts and toxicity of which often exceed the ability of the environment to clean itself. Therefore, the systematic analysis and monitoring of the environment is increasingly a matter of interest. Bee presence is considered an informative indicator of environmental health, but also the products and materials of their hives are considered good indicators of environmental contamination by toxic substances. Bees can carry many pollutants deposited on plants back to the hive; pesticides used in agriculture can become the cause of large-scale mortality for these insects.

Goal 12. Ensure sustainable consumption and production patterns: Bees are responsible for carrying out one of the most important processes in nature: the pollination of most plants. This work is essential to fertilize the flowers and ensure the production of viable fruits and seeds. In this way, bees help sustain the life of plants and other animals. Crops also benefit from receiving pollination services from bees, thus, ensuring their production; this has an impact on food security. Suitably raising native bees can promote increased crop yields and maintain a sustainable production of bee products for personal and local consumption.

Goal 13. Take urgent action to combat climate change and its impacts: Climate change has been one of the possible drivers of pollinator decline. Local declines in pollinator species richness and abundance, as well as geographic shifts in species distributions, have also been demonstrated. In addition, climate change can alter the phenology of flowering, negatively affecting pollinators by causing a spatial or temporal mismatch between plant and pollinator. Joining forces and undertaking actions that help mitigate the effects of climate change will have a positive effect on the populations of native bees and pollinators in general. We can support pollination services in wild plants and agroecosystems, thus benefiting our food security.

Goal 15: Land conservation: Bee populations are negatively affected by multiple causes, especially loss of natural habitats, which is generally the result of various human activities such as deforestation, agriculture, raising livestock, urbanization, and other practices that contribute to habitat fragmentation. Therefore, it is necessary to promote land conservation to maintain bee populations that play an essential role in our planet. An alternative to achieve this goal is to include strips of land with flowering plants around the crops in agricultural operations, which will attract a larger diversity and number of pollinators and prevent the destruction of too much land for agriculture.

Potential Implications for Decisions

1. Individual.
    a. When you observe a bee, are you now aware of the beneficial microbes associated with it?
    b. If you have a garden, would you like to plant flowers that you know will attract stingless bees? Would you use them as pollinators for an orchard at home?
    c. Would you eat other stingless bees products like bee bread and propolis? Would you consider using products or medicines containing stingless bees honey?

2. Community policies.
    a. Considering the benefits of commercial products derived from stingless bees, for example, honey used as wound dressings, can you think of any other possible/innovative product?
    b. Do you think a beehive would serve as a good model to improve teamwork and communication skills?
    c. Do you know of any policies on pollution, littering, deforestation, and agriculture that will protect green spaces that are natural habitats of stingless bees? If not, what do you think should be done to ensure this?

3. National policies relating to the topics.
    a. Do you know what Meliponiculture is? Now that you know what it is, do you believe that it is a good idea to encourage the implementation of Meliponiculture through educational programs and incentives for farmers to manage stingless bee's colonies?
    b. In which ways could the country promote pioneering and innovative studies about the microbiome of stingless bees to understand their function, and to develop products in the medical, pharmaceutical, and cosmetic industries?
    c. Could reducing the use of agrochemicals and establishing strips of land with flowering plants have a significant impact on stingless bees preservation?

Pupil Participation

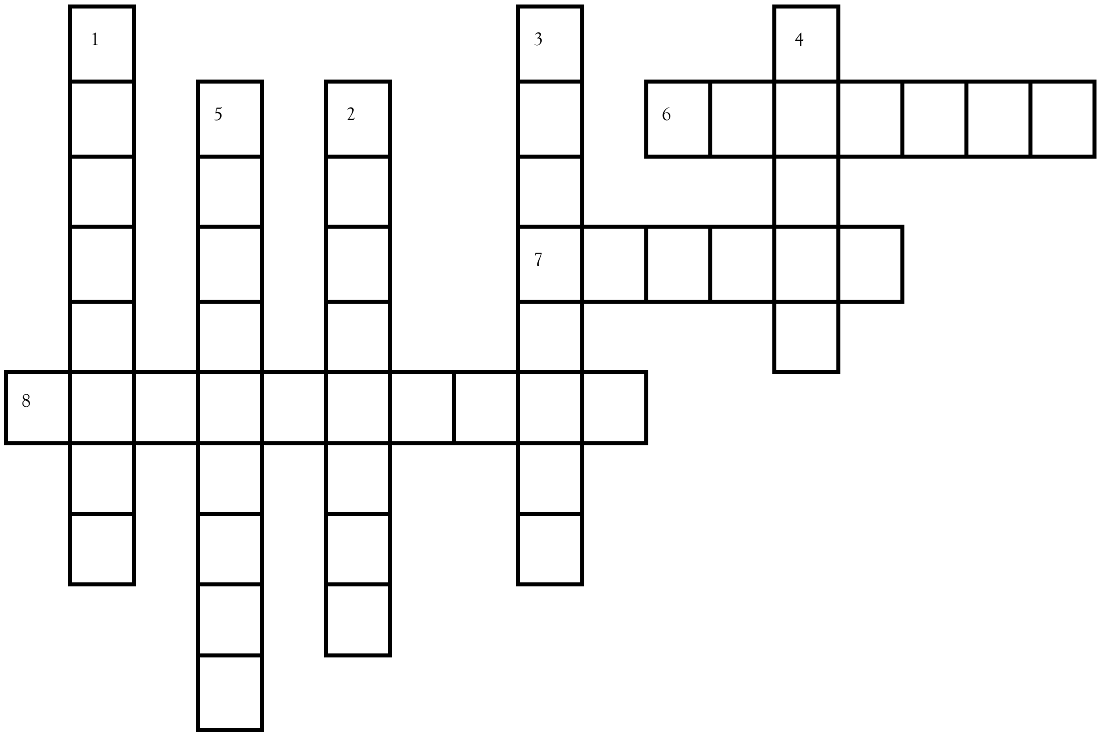
1. Class discussion of bee ecology, hive microbiome, and potential economic impact of stingless bees.
2. Pupil stakeholder awareness
    a. How has my perception of bees changed, knowing about the presence of microorganisms?
    b. Everything on the planet is interrelated, how does the decline or disappearance of stingless bee species affect humans?
    c. What is the key for bees organizing themselves efficiently in large groups?

3. Exercises: Teamwork activity
    a. Crazy hat
        i. Divide the players into teams of five members.
        ii. The teams temporarily adjourn to separate parts of the room.
        iii. Each member of the team will build a crazy hat representing a component of the hive (assigned by the teacher or chosen from a specific list) and their related microorganisms.
        iv. Once they have finished, the teams come back to the same part of the room wearing the crazy hats and explain them to the rest of their classmates.
        v. Their partners will carefully observe the crazy hats and will try to guess the participant's role.
        vi. At the end everyone will think about what happens if any of these components were not present in the hive.

    b. Crossword puzzle (Copyright 2022. Discovery education).

DOWN
1. One type of microorganism present in the stingless bee microbiome.
2. Barrier made of resin mixed with small amounts of salivary gland secretions and wax.
3. Regions of the world where stingless bees are found.
4. Extremely important colony component fermented with the contribution of *Bacillus* bacteria.
5. Multi-organismal body where most cells are microbial.

ACROSS
6. Structure formed by microorganisms infecting a wound that can be destroyed by components of the stingless bee honey.
7. Main nutrient source of the colony.
8. Products to ensure stingless bee colony health and immunity.



The evidence base, further reading, and teaching aids

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Glossary

Agrochemicals are chemicals used in agriculture, including chemical fertilizers, herbicides, and insecticides.
Antibiotics are medicines that destroy or inhibit the growth of bacteria.
Bee bread is a bitter, brownish mixture of nectar and pollen that is often prepared, stored in the hive, and used by bees as food for their larvae.
Biocontrol is the control of pests and parasites by using other organisms, e.g. control of mosquitoes by fish and other aquatic predators that feed on their larvae.
Biofertilizers are substances containing living micro-organisms which promote plant growth.
Biofilm is a community of microorganisms attached to a surface that produces a protective cover allowing them, in some cases, to infect wounds and undermine wound healing.
Brood chambers are structures where the larva develops.
Castes in eusocial insects are structurally and functionally specialized individuals, e.g. queen and workers.
Core microbiome refers to any set of microbial taxa, or the genomic and functional attributes associated with those taxa, that are characteristic of a host or environment of interest.
Diversity is the level of global or local biological diversity, often quantified crudely as numbers of species or of higher taxa; increasingly taken as indicative of genetic diversity level.
Ecosystem is a dynamic complex of organisms and their physical environment interacting as a functional unit.
Engineered is an organism modified by manipulation of its genetic material.
Environments are the surroundings or conditions in which a person, animal, microorganism, or plant lives or operates.
Eusocial species are any colonial animal species that live in multigenerational family groups in which most individuals (workers) cooperate to aid relatively few (or even a single) reproductive group members (queens).
Fermentation is a process by which yeasts or bacteria produce alcohol, lactic acid, acetic acid, and other chemical by-products as they metabolize sugars derived from varied sources.
Gland is an organ that manufactures chemical substances.
Holobiont is an assemblage of a host and the many microbes living in or around it, which together form a discrete ecological unit.
Immunity is the ability of an organism to resist infection.
Involucrum is a resistant material made of different proportions of wax and resin, which the bees build around the brood chambers.
Mayan Culture is an ancient pre-Columbian civilization known for its art, architecture, mathematics, and astronomy.
Meliponini is a tribe or group that is also known as stingless bees.
Meliponiculture is the breeding and management of native stingless bees.
Mesoamerican is the historical region of the American continent that comprises the southern part of Mexico to the northern part of Costa Rica.
Microbiome is the collection of genomes from all the microorganisms in a particular environment.
Microorganism is an organism that is too small to be seen by the unaided human eye.

Probiotic is a live microorganism that, when administered in adequate amounts, confers a health benefit on the host.
Resin is a sticky substance, insoluble in water, exuded by some trees and other plants.
Symbiont an intimate relationship between organisms, often developed through prolonged association and coevolution.
Wound dressing is a medical preparation that promotes the healing of wounds.
Wound healing is the biological process of replacement and reconstruction of damaged tissue.